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ATTACHMENT IV

TERATOGENIC STUDY IN RATS EXPOSED TO HALON 1301 BY INHALATION

Haskell Laboratory Report No. 499-78

Medical Research Project No. 2777

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TERATOGENIC STUDY IN RATS EXPOSED TO HALON 1301 BY INHALATION

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This study was conducted by Ms. Carole Doleba Crowe under the direction of Dr. H. J. Trochimowicz. Laparotomy, necropsy and gross pathology of the dams and gross examination of the fetuses were carried out by Dr. R. Culik, Mrs. Jean A. Hostetler, Ms. Ann S. Rogers, Mr. A. H. Stenholm, Mr. W. I. Swan and Mr. F. L. Ulmer. Clearing and Alizarin staining of the skeletons, and inspection of the fetuses were done by Dr. R. Culik, Ms. Ann S. Rogers, Ms. Alice V. Erwin, and Mr. A. H. Stenholm. Statistical evaluation of the data was carried out by Mr. W. E. Fayerweather. The above work was supervised by Dr. J. G. Aftosmis.

Rooms in which rats were housed were controlled for temperature (72-74° F) and humidity (50%). Food[1] and water were offered ad libitum except when exposed. During the 10-day exposure period the animals were fed one hour following exposure until exposure the next day.

Rats in the test groups were exposed to nominal concentrations of 1000, 10,000 or 50,000 ppm (v/v, in air) of Halon 1301 vapors on days 6 through 15 of gestation for 6 hours daily in 600-liter Rochester-type inhalation chambers. Control rats were exposed simultaneously to room air in a 1.4 m^3 stainless steel and glass dynamic chamber.

Rats were observed daily for clinical signs of toxicity and changes in behavior. They were weighed to within one gram on day of arrival, twice weekly thereafter and again on the day of sacrifice.

The desired vapor concentrations were obtained by adding a metered flow of Halon 1301 gas to the primary chamber air supply.

Chamber concentrations were routinely monitored at approximately one-half hour intervals using a Varian Aerograph Model 600D gas chromatograph equipped with a flame ionization detector. A 1' x 1/8" O.D. stainless steel column packed with 10% SE-30 on 60/80 mesh Chromosorb® W HP was maintained at 50°C for the analyses.

Design Levels	Analytical Results
Halon 1301	(Mean ± SD)
1,000 ppm	962 ± 57
10,000 ppm	10,196 ± 1,514
50,000 ppm	49,505 ± 4,753

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[1] Purina Rat Chow, Purina Company, St. Louis, Missouri

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INTRODUCTION

The purpose of this study was to evaluate the teratogenic potential of monobromotrifluoromethane (Halon 1301) when inhaled by pregnant rats during the period of fetal organogenesis.

TEST MATERIAL

Test material was a nonflammable compressed gas (colorless liquid at 25°C in cylinder) with a purity of 99.99 +%. It was submitted by Robert A. Gorski of the Petrochemicals Department. Other names for this product are FC-13B1 and FC 1301. It was assigned Haskell No. 12206, MR-2777-002.

PROCEDURE

Test animals were Charles River CD (ChR-CD) primigravida albino rats bred at Charles River Breeding Laboratories, Inc., North Wilmington, Mass. One-half of the rats were bred on April 17, 1978 and the other half on April 18, 1978. All rats were received in one shipment on April 20, 1978 as three and two days pregnant*. The morning sperm was found in the vagina was counted as Day 1 of gestation.

The 108 rats were randomly assigned to one of four exposure groups, each group consisting of two lots according to breeding date. There were 27 rats per group, housed individually in suspended stainless steel wire mesh cages and identified by cage card number.

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* All data are presented for the sum total as a unit.

All rats were sacrificed by chloroform inhalation on the twenty-first day of gestation. At sacrifice, the abdominal wall of the female was opened and both ovaries and uterus were removed and inspected. Next the uterus was opened and the fetuses removed and examined. The following observations and measurements were recorded:

1. Number of corpora lutea in each ovary
2. Number of implantation sites in each horn
3. Number and location of all live and dead fetuses.
4. Number and location of resorptions
5. Weight of each live fetus (to 0.01g)
6. Crown-rump length of each live fetus (to 0.01 cm)
7. Any gross anomaly which could be observed under a long focal length lens of 2½ x.

About one-half of the fetuses from each litter were preserved in 95% alcohol for subsequent maceration in 1% aqueous KOH, clearing and staining with Alizarin Red and examination to detect skeletal abnormalities. The remaining fetuses were fixed in Bouin's fluid for free-hand razor-blade sectioning by the Wilson method (1) with the modification described by Barrow and Taylor (2) and examination under a dissecting microscope for visceral and neural anomalies. Measurement of the lens of each eye was recorded as a part of this examination. The uterus and ovaries of rats in all groups were examined for gross changes and those of pregnant rats were preserved in Bouin's fluid for possible histologic examination. Other tissues and organs were examined grossly and discarded if found to be normal.

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(1) Wilson, J. G. (1965) Methods for administering agents and detecting malformations in experimental animals. In: Teratology: Principles and Techniques. J. G. Wilson and J. Warkany, eds., Univ. of Chicago, pp. 262-277.

(2) Barrow, Mark V. and Taylor, W. Jape (1969). A rapid method for detecting malformations in rat fetuses. Journal of Morphology, 127:291-306.

STATISTICAL EVALUATION

For statistical evaluation of the data, the litter was considered the experimental unit of treatment and observation. The Fisher Exact Probability test was used to evaluate the incidence of resorptions and abnormalities among litters. Maternal and fetal body weights and measurements were treated statistically by analysis of variance and least significant difference (LSD) tests. The number of corpora lutea, implantations and live fetuses per litter were analyzed by the Wilcoxon rank sum test. In all cases two tailed significance tests were performed and significance was judged at the 0.05 probability level.

RESULTS

1. Body Weight

No meaningful differences in average body weight gains were found between the control and any test group.

2. Clinical Signs

Minor hair loss occurred in two rats, one each in the 0 and 50,000 ppm groups. Two rats in the high level (50,000 ppm) developed brown crusty sores. One rat in the high level group was blind in the right eye on arrival. She was removed from the test for further observation and is not included in the final tabulation of the data.

3. Gross Changes in Organs of Dams at Necropsy

No gross pathological changes were observed in the ovaries and uterus or vital organs and tissues of pregnant treated females.

4. Pregnancy Outcome and Fetal Development

A summary of the effect of exposure to Halon 1301 on the dams, and on the outcome of pregnancy and fetal development is presented in Table I.

Although the mean body weight of pregnant females in the high-level group was lower than the mean of the control group, the difference was not statistically significant and the actual gains in body weight were similar.

Parameters measuring the outcome of pregnancy and fetal development, including the number of litters with partial and total resorptions, and the body measurements of the fetuses were not different from those of the concurrent controls.

5. Fetal Anomalies and Malformations

The type and the incidence of external, visceral and skeletal anomalies and malformations among litters in the control and treated groups and the number of fetuses examined are summarized in Table II.

Small subcutaneous hematomas and petechial hemorrhages on various parts of the body were found in fetuses from litters of all groups including the control group. Two runts were found, one in the control group and one in the intermediate level.

Visceral examination showed a slight incidence of hydronephrosis and undescended testes in all groups and a very slight incidence of hydroureter in the control and the mid-level groups. One fetus in the control group was found with hydronephrosis and hydrocephalus. In the group receiving 10,000 ppm Halon 1301, one fetus showed hydrocephalus plus a herniated diaphragm, one had a blood-filled peritoneum, and another showed hydronephrosis and inguinal hernia. All three fetuses were from different litters.

No malformations or major abnormalities of the skeletal system were noted. The small incidence of minor anomalies and common variants listed in the table was similar in all groups. These variations are

biological and are related to chronological age of the fetuses (delayed ossification, bipartite centra) or to their genetic background (wavy and 14th full or rudimentary ribs) and not to the treatment.

SUMMARY AND CONCLUSIONS

1. Exposure of pregnant rats to Halon 1301 vapors from day six through fifteen of gestation at levels of 1000, 10,000 and 50,000 ppm had no effect on their body weight gains. No compound-related clinical signs of toxicity or changes in behavior were noted.

2. The outcome of pregnancy measured by the number of implantation sites, resorptions and live fetuses, was not adversely affected by the exposure.

3. Exposure did not affect embryonal development as measured by weight and crown-rump length of the fetuses.

4. Only three fetuses, each one from a different litter, were found with malformations. All three were from dams exposed to the intermediate level (10,000 ppm) of Halon 1301. These defects were not treatment-related. They are considered as spontaneous, congenital malformations of genetic origin seen in this strain of rats at about the same frequency as in this study.

5. Under the conditions of this test, Halon 1301 was not embryotoxic or teratogenic when inhaled by pregnant ChR-CD rats.

TABLE I

EFFECT OF HALON 1301 ON THE OUTCOME OF PREGNANCY AND FETAL DEVELOPMENT OF THE RAT

	Air Concentration of Halon 1301[1]			
	0 ppm	1000 ppm	10,000 ppm	50,000 ppm
Females bred	27	27	27	26
Females pregnant (%)	21 (77.8)	23 (85.2)	23 (85.2)	22 (84.6)
Corpora lutea/pregnant female	12.8 ± 3.2	13.4 ± 1.9	13.5 ± 2.7	12.5 ± 2.2
Implantations/litter[2]	10.7 ± 2.6	10.5 ± 2.1	10.9 ± 1.4	10.0 ± 2.7
Live fetuses/litter[2]	9.9 ± 2.7	10.0 ± 2.1	10.1 ± 1.7	9.5 ± 2.7
Litters with early resorptions (%)	12 (57.1)	7 (30.4)	11 (47.8)	10 (45.5)
Litters with late resorptions (%)	1 (4.8)	0 (0)	1 (4.3)	0 (0)
Litter with dead fetuses (%)	0 (0)	0 (0)	0 (0)	0 (0)
Litters with partial resorptions (%)	13 (61.9)	7 (30.4)	12 (52.2)	10 (45.5)
Litters totally resorbed (%)	0 (0)	0 (0)	0 (0)	0 (0)
Resorptions/litter with resorptions[2]	1.2 ± 0.7	1.6 ± 0.6	1.4 ± 1.1	1.2 ± 0.4
Intitial body weight of pregnant female (g)[2]	223 ± 11	225 ± 13	223 ± 12	219 ± 13
Final body weight of pregnant female (g)[2]	375 ± 38	380 ± 23	375 ± 25	365 ± 20
Fetal crown-rump length (cm)[3]	4.2 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	4.2 ± 0.3
Fetal weight (g)[3]	3.4 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.4 ± 0.1

(1) Administered by inhalation, 6 hrs./day on days 6 through 15 of gestation; sacrificed on day 21.

(2) Mean ± 1 standard deviation.

(3) Mean of litter means ± 1 standard deviation.

TABLE II

INCIDENCE OF ANOMALIES IN LITTERS (FETUSES) AFTER MATERNAL EXPOSURE TO HALON 1301(1)

Types of Anomalies	Concentrations			
	0 (Control)	1000 ppm	10,000 ppm	50,000 ppm
Gross				
No. litters (fetuses) examined	21 (207)	23 (231)	23 (233)	22 (208)
Petechial hemorrhage	10 (15)	8 (14)	8 (14)	3 (5)
Subcutaneous hematoma	3 (4)	8 (9)	5 (8)	6 (6)
Agnathia	-	-	1 (1)	-
Runts	1 (1)	-	1 (1)	-
Visceral				
No. litters (fetuses) examined	21 (100)	23 (109)	23 (111)	22 (101)
Hydronephrosis	2 (2)	3 (3)	3 (3)	2 (2)
Undescended testes	2 (2)	2 (2)	3 (4)	4 (4)
Hydroureter	1 (1)	-	1 (2)	-
Hydronephrosis and hydrocephalus	1 (1)	-	-	-
Liver peliosis	4 (4)	1 (1)	3 (3)	-
Peritoneum - fluid filled	-	-	1 (1)	-
Hydronephrosis and inguinal hernia	-	-	1 (1)	-
Skeletal				
No. litters (fetuses) examined	21 (107)	23 (122)	23 (122)	22 (107)
14th Rudimentary rib(s)	16 (45)	21 (60)	21 (69)	20 (56)
Sternebrae unossified	5 (9)	11 (13)	9 (11)	12 (20)
Centra bipartite	2 (2)	2 (2)	1 (1)	2 (2)
14th Rib(s)	-	3 (3)	1 (1)	2 (3)
Wavy rib(s)	-	2 (2)	3 (3)	3 (3)

(1) Administered by inhalation, 6 hrs./day on days 6 through 15 of gestation; sacrificed on day 21.